

A chemiluminescent substrate of hydrolytic enzyme having the following general Formula I, as follows:

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where "Lumi" is a chemiluminescent moiety capable of producing light (a) by itself, (b) with MP attached and (c) with M attached. Lumi includes, but is not limited to, chemiluminescent acridinium compounds (e.g. acridinium esters, acridinium carboxyamides, acridinium thioesters and acridinium oxime esters) benzacridinium compounds, quinolinium compounds, isoquinolinium compounds, phenanthridinium compounds, and lucigenin compounds, or the reduced (e.g., acridans) or non-N-alkylated forms (e.g., acridines) of the above, spiroacridan compounds, luminol compounds and isoluminol compounds and the like. M is a multivalent heteroatom having at least one lone pair of electrons selected from oxygen, nitrogen and sulfur, directly attached to the light emitting moiety of Lumi at one end and to P at the other end. (When M alone is attached to Lumi to form Lumi-M, it does, of course, have either a proton or a counterion associated with it or is in the form of an ion.). P is a group that can be readily removed by hydrolytic enzymes, as discussed in more detail hereinafter. The light emitting moiety of Lumi is well known. For example, when Lumi is an acridinium compound or luminol, the light emitting moiety

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$$\text{Lumi-M-P} \xrightarrow[\text{Reaction A}]{\text{HE}} \text{Lumi-M} + \text{P}$$

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